Plastocyanin Silencing will Decrease PVXCP Accumulation in Chloroplasts of Tobacco Plant

Chan C.M.¹ and Wong S.M.²

Department of Biological Sciences, The National University of Singapore
Blk S2, 14 Science Dr 4, Singapore 117543.

ABSTRACT

Plastocyanin is an important component in photosynthesis and biogenesis of chloroplast. It is a nucleus-encoded protein and transports into chloroplast with the help of transit peptide. PVXCP interacts with plastocyanin transit peptide in the transportation of PVXCP into chloroplast. A research was conducted to investigate the interaction of plastocyanin and PVXCP. Virus induce gene silencing approach had been used to silent plastocyanin in tobacco plant from the beginning. Plastocyanin silencing mRNA was inserted on TRV2 plasmid of Tobacco mosaic virus. TRV1 and TRV2 were transformed into Agarobacteria separately and the clones will be agroinfiltrated into the young leaves. The efficiency of PVXCP transporting into chloroplast and amount of PVXCP accumulated in various parts of chloroplast in plastocynin-silenced plant was examined and confirmed by using western plot. In second part of this project, pBI121-PVXCP was cloned by using cloning method. The pBI121-PVXCP will express PVXCP without the presence of PVX in tobacco plant and level of PVXCP accumulated in chloroplast can be determined in future work.

INTRODUCTION

In this study, I have two specific objectives which I hope to achieve. First, I wish to know about the interaction of plastocyanin and PVXCP using virus-induced gene silencing approach. Second, I also wish to determine whether the relative amount of PVXCP in various parts of chloroplast in control and plastocyanin-silenced plants varies. In second part, I hope to obtain a recombinant plasmid pBI121-PVXCP which was used in the future work on determining the PVXCP level in chloroplast of plastocyanin-silenced plants without PVX infection.

MATERIALS AND METHODS

This study was conducted using various methods: virus-induced gene silencing, chloroplast isolation and fractionation, protein extraction, western blot and gene fusion. Virus-induced gene silencing was used to silent plastocyanin expression in tobacco plant by using TRV virus vector and protein extraction was carried out to examine the effect of silencing. Chloroplast isolation and fractionation were carried out to extract the PVXCP from various compartments of chloroplast.

¹ Student
² Supervisor
Besides that, western blot was used to compare and determine the amount of PVXCP and plastocyanin. In second part of the project, gene fusion approach was used to produce a recombinant plasmid pBI121-PVXCP.

RESULTS

Expression of Plastocyanin

Expression of plastocyanin on the young leaves was examined after 12 days post agroinfiltration with western blot using plastocyanin antibodies. The result from western plot shows that there is no plastocyanin expression in gene silencing plant. This suggests that plastocyanin had been silenced successfully.

Accumulation of PVXCP Decreased in Chloroplast

From the result on western blot, the accumulation of PVXCP was reduced in various parts of chloroplast in plastocyanin-silenced tobacco plants compared to control plants after 14 days post inoculation. These strongly proved that the accumulation of PVXCP in chloroplast is dependant on plastocyanin. Its entry into chloroplasts may be facilitated by binding to the transit peptide of plastocyanin.

Plasmid Construction

PCR amplification was carried out to amplify PVXCP fragments. Restriction digestion for PVXCP fragments and vector pBI121-CYP1 using BamHI and Sac1 were performed to create sticky ends. pBI121-PVXCP was obtained by ligating pBI121 and PVXCP. On the gel electrophoresis, the band of 700bp shows that there is an insert fragment on the recombinant plasmid.

DISCUSSION

Plastocyanin Expression

From the result, we know that there are different sizes of plastocyanin in plant. Precursor plastocyanin is the larger molecule with full length of transit peptide. However, precursor plastocyanin is unable to be detected in western blot because it normally in cytoplasm. Intermediate plastocyanin is smaller than precursor plastocyanin as it lacks N-terminal of transit peptide. It is normally found in stroma where the N-terminal had been cleaved by SPP. Mature plastocyanin which was found in lumen has the smallest size where the transit peptide had been cleaved off.

Accumulation of PVXCP Decreased in Chloroplast

We had identified the interaction between PVXCP and plastocyanin by presenting data that suggested that plastocyanin played an important role in assisting the PVXCP translocation. The reduction of PVXCP level in the absence of plastocyanin suggested that the transportation of CP into chloroplast depends on binding with plastocyanin transit peptide. Some coat protein may still be able to transport into chloroplast in the plastocyanin-silenced plants because virus-induced gene
silencing approach does not silent the expression of protein in the plant completely. This is due to the virus unable to invade every cell.

Plasmid Construction

Cloning efficiencies were invariably proved to be very low as there were only several colonies grown on the Kan+ plate. It is believed that this was due to the large size of vector that caused the presence of excess damaged plasmids during the purification. Besides that, the large vectormolecules with sticky ends may become linked or self ligated. Self ligation is due to the imperfect restriction digestion of pBI121 vector by either one of the digestion enzyme.

REFERENCES


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